Oral colistin sulfate in pigs: pharmacokinetics and effect on fecal *Escherichia coli* excretion of weaned pigs challenged with *Escherichia coli* F4 (K88)

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Abstract

Colistin sulfate (CS), a polymyxin antibiotic, is used in Canada for the treatment of post-weaning diarrhea in pigs as an alternative to neomycin. The aim of the present study was to evaluate some pharmacokinetics parameters of CS and its effect on the evolution of the intestinal *Escherichia coli* population in pigs challenged with enterotoxigenic *E. coli* (ETEC): F4. A total of 14 weaned piglets were divided into two groups, a non-challenged, treated group (n=7) and a challenged, treated group (n=7). Both groups received a single oral dose of CS at 50,000 IU/Kg. Challenge was carried out by oral administration of 10^9 CFU of a hemolytic ETEC: F4 strain resistant to nalidixic acid. Blood samples were taken at 30 min and 1, 2, 4, 6, 8, 12, 24, 36 and 48 hours post treatment from each pig and CS quantification was performed by LC-MS/MS. In the challenged group, severity of diarrhea was monitored and the presence of the ETEC:F4 strain in the feces was enumerated using 5% bovine blood agar plates containing nalidixic acid. In both groups, total *E. coli* counts were carried out using Petrifilm *E. coli*/Coliform count plates. In both groups, the plasma concentration of CS was below the lower limit of quantification (20 ng/ml). Following CS treatment, a decrease in the total *E. coli* and ETEC: F4 fecal counts were observed at 24 h post treatment. The fecal consistency was not affected by CS treatment.

For the first time, a study of some CS pharmacokinetics parameters with a highly sensitive method showed that CS levels are not detectable in systemic circulation following oral administration, and concurrent oral challenge with an ETEC strain did not affect CS absorption. A single dose of CS resulted in reduced bacterial counts of the total *E. coli* and ETEC: F4 populations in the feces.

Introduction

Colistin (polymyxin E), a cationic antimicrobial peptide produced by *Bacillus polymyxa subsp. Colistinus.* This antibiotic is used in Canada for the oral therapy of intestinal infections in pigs, particularly those caused by *E. coli.* Colistin is used clinically in the form of its water-soluble colistin sulphate (CS) (Chauvin, Beloeil, Orand, Sanders, & Madec, 2002). In pigs receiving therapeutic doses by the oral route, it was found that CS was poorly absorbed, plasma concentrations being less than the lower limit of quantitation (0.250 µg/mL) of high-pressure liquid chromatography (Guyonnet et al., 2010).

The objective of the present study was to develop an analytical technique with high sensitivity for monitoring plasma concentrations of CS following oral administration, and to determine the effect of experimental infection with enterotoxigenic *E. coli* (ETEC):F4 (K88) on CS absorption level. And we also determined the effect of an oral dose of colistin (50.000 UI/kg), on the level of fecal shedding of total *E. coli* and of ETEC: F4 (K88).

Materials and methods

1. **Animals**

Fourteen clinically healthy piglets, 21 days of age at the beginning of experimentation, selected for the presence of F4 gene by PCR-RLFP were used in this study. Each pig was individually housed in a pen, fed a standard non-medicated ration for post-weaning pigs and received water *ad libitum*. Piglets were weighed at the beginning and end of the experiments. The temperature was kept at 24–26°C. Animals were allowed to acclimatize for 2 days before beginning of experiments.

2. **Jugular catheterization of pigs and blood sampling**

After the 2 days of acclimatization (23 d old), animals were restrained on a V-shaped table, and a catheter was inserted over the wire guide in the jugular vein as previously described (Matte, 1999). Blood samples (3mL) from the catheter were collected into EDTA tube, from one day after catheter placement (24 d old) until animal's euthanasia (32 d old). After CS oral administration (30 d old), blood samples (3mL) were collected, 30 min and 1, 2, 4, 6, 8, 12, 24, 36 and 48h following

CS administration. These samples were used to determine CS plasma concentrations by LC-MS/MS.

3. Experimental infection, antibiotic administration and health status

The challenge strain for experimental infection of pigs, was a nalidixic acid-resistant (Nal^r) variant of ETEC F4 strain ECL8559 (0149:LT: STa: STb: East1: paa: hemβ: F4), as previously described (Daudelin et al., 2011). At 27 days of age, 7 pigs were each orally challenged with 5 mL of trypticase soy broth containing 10^9 CFU of the ETEC F4 challenge strain following the administration of 10 mL $CaCO_3$. At 30 days of age, each pig of the two groups of piglets received a single oral dose of colistin sulfate (50.000 IU/kg). Clinical examination included observation of fecal consistency, behavioural disturbances and presence of cyanosis. The severity of diarrhea was quantified by using a fecal consistency scoring (0, normal; 1, soft feces; 2, mild diarrhea; and 3, severe diarrhea).

4. Analytical methods

A high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for CS quantification in plasma following an oral administration. The HPLC system was a Perkin–Elmer liquid chromatography apparatus (Series 200, Boston, MA), and the spectrometry system used was an API 2000 QTRAP (AB-Sciex, Concord, Canada). CS was extracted from pig's plasma by using a protein precipitation method. The lower limits of quantitation (LLOQ) of the method were 20 ng/mL of plasma. Chromatographic separation and the mass spectrometry detection were derived and optimized based on (Ma, Wang, Gerber, & Milne, 2008).

5. Microbiological analysis of fecal samples

Faecal samples were collected before challenge and 24, 48, 72, 84, 96, 108, 120 h post challenge. These samples were used to examine the presence of the challenge strain ETEC: F4 strain and total *E. coli* population. A quantity of 10 g of fecal samples was diluted 10-fold in peptone water and selected dilutions were plated on to Petrifilm *E. coli*/Coliform count plates, and 5% bovine blood agar plates, containing nalidixic acid, for counting of the total *E. coli* population and the haemolytic challenge ETEC F4 strain respectively. The plates were incubated aerobically for 24 h at 37°C.

Results

1. Quantification of plasmatic colistin sulfate concentration

For the two treated groups (infected and not infected), in all samples, the plasma concentration of CS was less than the lower limit of quantitation (20ng/mL). In the non-infected treated group the concentration of CS was greater than the limit of detection (LOD) after 30 minutes of CS administration but less than LLOQ (Figure 1). However, in the non-infected treated group, at all sampling times, the concentration of CS was less than the LOD (figure 2).

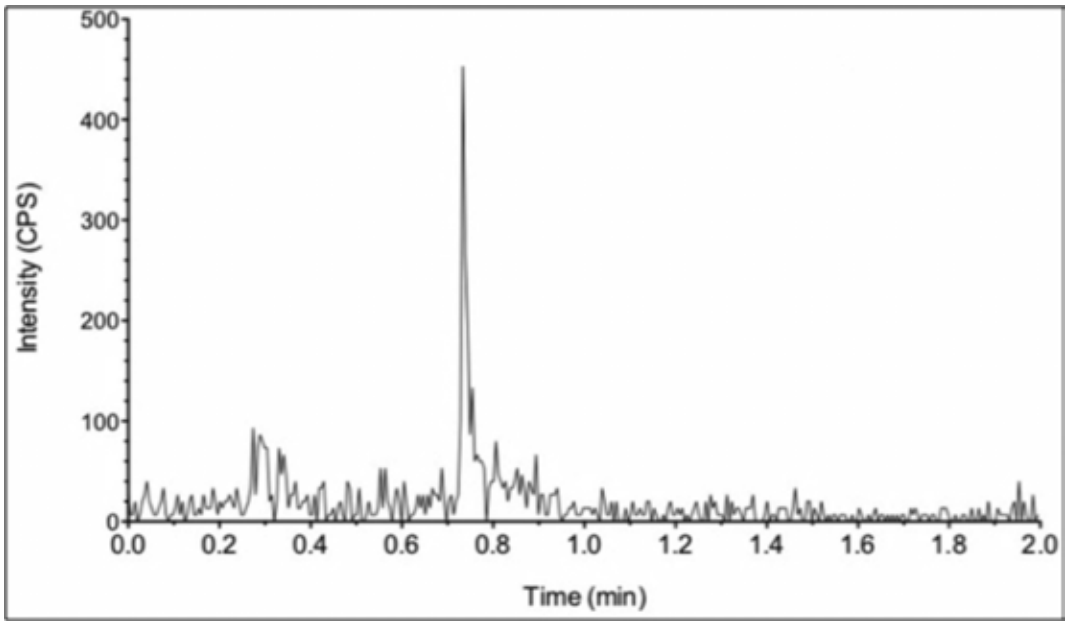

Figure 1: LC-MS/MS mass chromatogram of a typical sample from the non-infected group at 30 min following CS administration. Plasmatic concentrations of CS were above the limit of detection but significantly less than the limit of quantitation (20ng/mL). CS was not detected at other time points.

2. Analysis of Bacterial Shedding and Clinical Scoring of Diarrhea

None of the animals showed clinical diarrhea or had to be removed from the experiment due to illness. However, one animal in the infected treatment group was not used due to poor feed intake, and was consequently removed from the experiment. The mean number of ETEC: F4 colonies recovered from the faeces of the 6 experimentally challenged treatment pigs is shown in Figure 3. Experimental challenge was performed at 0 hour, and treatment with CS was carried out at 72h post challenge. The administration of a single oral dose of CS reduced mean fecal ETEC F4 counts during the next day following treatment; with a maximum effect at 24h post-treatment (96h post challenge). Nevertheless, the mean count following treatment was not significantly lower than the of baseline values (72h post challenge). At 24h post treatment, the fecal ETEC F4 numbers were increased to regain baseline values (Figure 3). In both treated groups, that is in challenged and non- challenged animals, total *E. coli* counts demonstrated the same trend of decline as observed for ETEC F4 (i.e maximum effect was observed at 24h post-treatment (96h post challenge). The highest mean diarrhea score of the challenged-treated groups was observed at 72h post challenge. After CS treatment, there was a tendency for score of diarrhea to decrease, with a maximum effect observed at 48h post treatment (120 h post challenge).

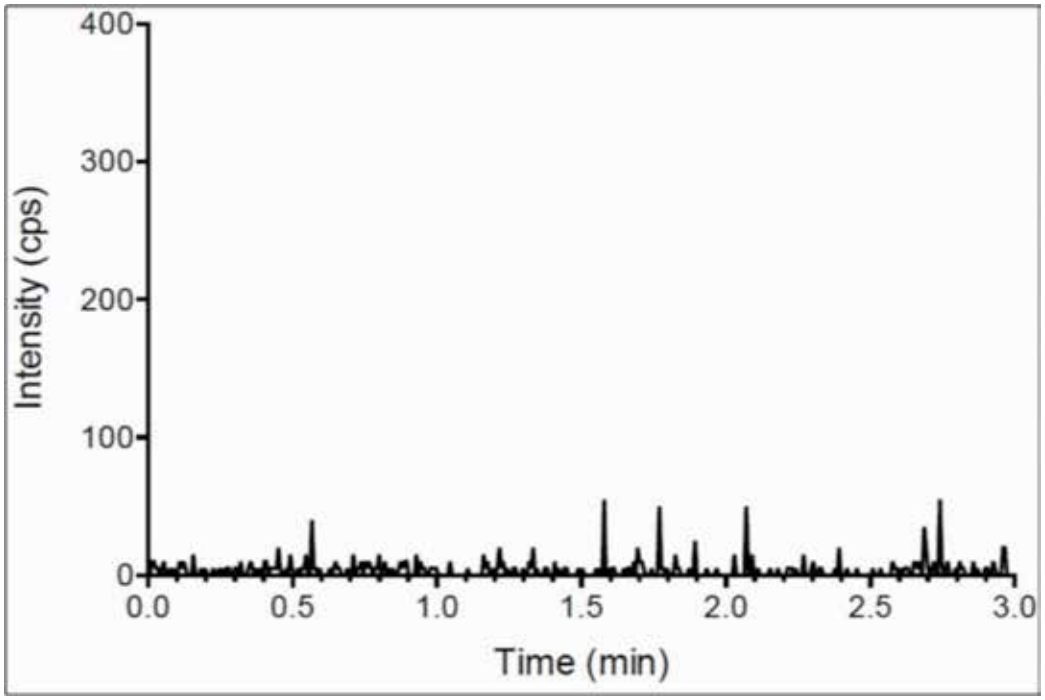

Figure 2: LC-MS/MS mass chromatogram of a typical sample from the infected group at 30 min following CS administration. Plasmatic concentrations of CS were less than the limit of detection. Furthermore, CS was not detected at other time points.

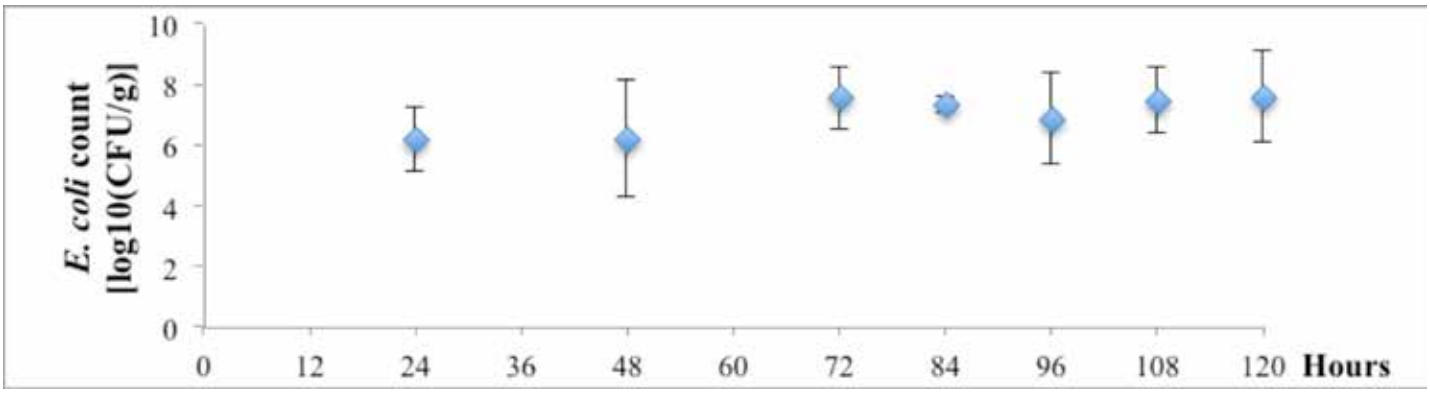

Figure 3: Evolution of the fecal ETEC F4 count (means, and standard deviation). Experimental inoculation was performed at 0 hour, and treatment with CS was carried out at 72 hours post challenge. Maximum effect of CS was observed at 24h post-treatment (96h post challenge).

Discussion

To the best of our knowledge, non other experimental studies focussing on CS systemic residues in pigs using LC-MS/MS as an analytic method. In our study, healthy pigs showed a very small level of systemic CS at 30 minutes after its oral administration, and these quantities were lower then the LLOQ. Thus, the absence of CS absorption in healthy pig after oral administration despite the use of a sensitive analytic method (LOQ=20 mg/ml), confirms the reports of previous workers

who demonstrated that colistin was poorly absorbed and systemic residue levels were usually undetectable (Guyonnet et al., 2010). In challenged treated group, CS systemic concentrations were not detected in any of the samples analyzed. These results differ from those of studies that have shown that infectious gastroenteritis increases gut permeability, and inflammation appears to act via mast cells, to an increase intestinal permeability (Camilleri, Madsen, Spiller, Greenwood-Van Meerveld, & Verne, 2012). In our study, the enterotoxins produced by the ETEC: F4 strain cause secretion of water and electrolytes leading to diarrhea with few microscopic lesions (Fairbrother & Gyles, 2012).Thus, diarrhea and the effect of enterotoxins may explain the non detection of CS systemic concentration. Our study showed that antibacterial effect of CS in bacterial shedding was most important in the 24-h post treatment period, beyond this range, its antimicrobial activity being absent. Thereby, one dose per day of CS is ineffective to produce significant dimunition of bacteria counts.

Conclusion

For the first time, a study of some CS pharmacokinetics parameters with a highly sensitive method showed that CS levels are not detectable in the systemic circulation following oral administration in pigs, and concurrent oral challenge with an ETEC F4 strain did not affect CS absorption. A single dose of CS resulted in reduced bacterial counts of the total *E. coli* and ETEC: F4 populations in the feces. Further studies are needed to evaluate the effect of CS on *E.coli* populations and potential antimicrobial resistance.

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